Hailey–Hailey Disease and Calcium: Lessons from Yeast

To the Editor:

Hailey–Hailey disease is an autosomal dominant skin disorder leading to acantholysis of the epidermis. The disorder has been linked to mutations in *ATP2C1* (Hu *et al*, 2000). *ATP2C1* is the human homologue of the *Saccharomyces cerevisiae PMR1* (plasma membrane ATP-ase related), which encodes the first characterized member of the novel Golgi-associated secretory pathway Ca^{2+} transport ATPases (SPCA). Accordingly, the protein product of the human *ATP2C1* is designated as hSPCA1. hSPCA1 fully complements the phenotype of the *S. cerevisiae pmr1*Δ mutant showing that the two proteins are functionally equivalent (Ton *et al*, 2002). This observation suggests that *pmr1*Δ mutants may serve as experimental models of Hailey–Hailey disease.

Previous reports demonstrated that hSPCA1 localizes to the Golgi apparatus controlling Ca^{2+} stores there, and that Hailey–Hailey keratinocytes react abnormally to increased extracellular Ca^{2+} levels (Aronchik *et al*, 2003; Behne *et al*, 2003). These findings suggest that altered Ca^{2+} homeostasis is a key element in the development of this disorder.

Recalcitrant Hailey–Hailey disease can be successfully treated with calcineurin inhibitors such as topical tacrolimus (FK506) and cyclosporine (Ormerod *et al*, 1991; Rabeni and Cunningham, 2002). While the major mechanism of action of these drugs is presumably the suppression of lymphocyte activation, a nonimmunomodulatory mechanism has also been proposed (Ormerod *et al*, 1991; Rabeni and Cunningham, 2002). To explore this direct mechanism, we studied the effects of calcineurin inhibition in *S. cerevisiae pmr1*Δ mutants.

*Pmr1*Δ mutants are sensitive to both low and extremely high extracellular Ca^{2+} levels of the growth medium (Miseta *et al*, 1999a; Kellermayer *et al*, 2003). Cyclosporine, a potent inhibitor of yeast calcineurin along with FK506 (Foor *et al*, 1992), has been recently shown to augment the low-extracellular-Ca^{2+} sensitivity of a *pmr1*Δ mutant (Kellermayer *et al*, 2003). To test the effects of calcineurin inhibition in the *pmr1*Δ mutant under Ca^{2+} stress, its growth was followed on agarose plates supplemented with Ca^{2+} in the presence and absence of cyclosporine (Fig 1). Calcineurin inhibition induced tolerance to high extracellular Ca^{2+} environment in the *pmr1*Δ strain through VCX1 (vacuolar Ca^{2+}/H^{+} exchanger) activity. This transporter decreases cytoplasmic Ca^{2+} stress by utilizing the pH gradient of the vacuole (Miseta *et al*, 1999b). Calcineurin inhibition has been found to augment VCX1 function in a post-translational way in *pmc1*Δ (vacuolar Ca^{2+}-ATPase) yeast. The growth of this strain was promoted under Ca^{2+} stress in *cnb1/pmc1* double mutants and following FK506 (tacrolimus) treatment (Cunningham and Fink, 1996). Our findings with cyclosporine are in accordance with these results and demonstrate that the role of calcineurin in the calcium tolerance of Ca^{2+}-

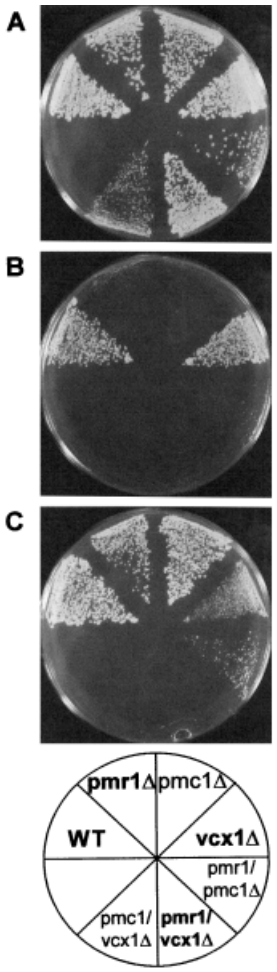

Figure 1
Cyclosporine (calcineurin inhibition) rescues the *pmr1*Δ mutant in high extracellular calcium environment through VCX1. *Saccharomyces cerevisiae* strains (clockwise: *WT*/wild-type/: SEY6210; *pmr1*Δ: YDB279; *pmc1*Δ: YDB224; *vcx1*Δ: YDB225; *pmr1/pmc1*Δ: YDB276; *pmr1/vcx1*Δ: YDB289; *pmc1/vcx1*Δ: YDB254 for precise genotypes see reference Kellermayer *et al*, 2003) were grown on agarose plates containing: (*A*). YPD buffered with 40 mM Mes-Tris to pH 5.5 (*B*). 500 mM Ca^{2+} added (*C*). 500 mM Ca^{2+} and 20 μg per mL cyclosporine added. The plates are representative and were made following a minimum of 2 separate observations of growth under similar circumstances in the case of each strain. While the growth of the *pmr1*Δ mutant is inhibited in 500 mM Ca^{2+}, cyclosporine treatment restores its growth through VCX1 activation, since the *pmr1/vcx1*Δ mutant cannot be rescued in the same environment. The figure also shows that PMC1 (vacuolar Ca^{2+}-ATPase) does not play a role in this respect, since the growth of the *pmr1/pmc1*Δ mutant is also restored by cyclosporine treatment.

ATPase mutants depends on VCX1 function (Fig 1). Observing the growth of the *pmr1*Δ and the *pmr1/vcx1*Δ mutant in liquid media containing high concentrations of Ca^{2+} (YPD supplemented with 40 mM Mes-Tris pH 5.5 and 400 mM Ca^{2+}) further supported the findings on agarose plates. Growth of the strains (A_{600}) was assessed after 12 h as percentage of the *WT* cultured in the same circumstances in two separate experiments and averaged according to Ton *et al* Cyclosporine (20 μg per mL) augmented the growth of the *pmr1*Δ in high extracellular calcium environment (from 58% to 97% compared to *WT*), but had no effect on the *pmr1/vcx1*Δ strain (62% and 65% without and with cyclosporine respectively).

*Pmr1*Δ mutants are not only sensitive to low and extremely high calcium levels, but are also susceptible to manganese (Lapinskas *et al*, 1995). Indeed, both PMR1 and hSPCA1 function as a Ca^{2+}/Mn^{2+} ATP-ase and share similar transport characteristics (Ton *et al*, 2002; Fairclough *et al*, 2003). However, the role of calcineurin in manganese tolerance depends on PMR1 function. Consistently, FK506 treatment had no significant effect on the Mn^{2+} sensitivity of *pmr1*Δ and *pmr1/vcx1*Δ mutants (Cunningham and Fink, 1996).

*Pmr1*Δ strains are also tolerant to sodium and are susceptible to oxidative stress (Lapinskas *et al*, 1995; Park *et al*, 2001; Ryu *et al*, 2003). However, calcineurin inhibition abolishes the salt-tolerant phenotype of the *pmr1*Δ mutant (Park *et al*, 2001). Furthermore, cyclosporine and tacrolimus treatment induced oxidative stress in human studies (Calo *et al*, 2002), suggesting that these drugs are unlikely to salvage cells sensitive to such stress.

In conclusion, among the various phenotypes of the *pmr1*Δ mutant, calcineurin inhibition is supportive only in extremely high Ca^{2+} environment. Calcineurin has been shown to be functionally active in human keratinocytes (Al Daraji *et al*, 2002). Based on the observations in yeast, calcineurin inhibitors such as tacrolimus and cyclosporine may be beneficial in the treatment of Hailey–Hailey disease by decreasing Ca^{2+} stress in epidermal cells. Lysosomal calcium stores are regulated in a pH-dependent manner in mammals (Christensen *et al*, 2002). These organelles could potentially play a similar role in the intracellular calcium homeostasis of higher vertebrate cells as vacuoles do in yeast. However, intracellular Ca^{2+} stress is only one aspect of Hailey–Hailey keratinocytes. Disrupted protein processing due to depletion of ions in the Golgi apparatus and Mn^{2+} toxicity as a result of hSPCA1 dysfunction remain additional problems to be addressed in order to reach a complete understanding and optimal treatment of this disorder.

Reka Szigeti* and Richard Kellermayer†
*Department of Dermatology at the University of Pécs, Pécs, Hungary;
†Department of Medical Genetics and Child Development at the University of Pécs, Pécs, Hungary

The authors would like to thank Dr David M. Bedwell and Dr Attila Miseta for providing strains and media, and Ibolya Lamár, for her assistance.

DOI: 10.1111/j.0022-202X.2004.23413.x

Manuscript received February 5, 2004; revised April 26, 2004; accepted for publication May 21, 2004

Address correspondence to: Richard Kellermayer, MD, Department of Medical Genetics and Child Development at the University of Pécs, Hungary, Jozsef A. u. 7., Pécs, 7623 Hungary. Email: richard.kellermayer@aok.pte.hu

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